# METHOD FOR DNA DOUBLE-STRAND BREAK REPAIR IN VITRO AND APPLICATIONS THEREOF

## CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional application of the pending U.S. non-provisional application Ser. No. 12/796,258 which was filed on Jun. 8, 2010. This application also claims priority from U.S. provisional patent application Ser. No. 61/185,868 and U.S. provisional patent application Ser. No. 61/268,255, both of which were filed on Jun. 10, 2009 and are incorporated by reference herein.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under 2007-DN-BX-K1 46 awarded by the US Department of Justice and GM067085 and GM032335 awarded by the National Institutes of Health. The government has certain rights in the invention.

## BACKGROUND

In cells of all organisms, DNA double-strand breaks are repaired by a process involving the action of RecA-class recombinases, helicases, nucleases, DNA polymerases, and DNA ligases. A classical pathway for double-strand break repair, called synthesis-dependent single strand annealing (SDSA), is shown in FIG. **1**. Briefly, nucleases and helicases are used to unwind the DNA at the broken end and degrade the 5'-ending strand. The region of single-stranded DNA (with a terminal 3' end) thus created is bound by a recombinase. The recombinase promotes a DNA strand invasion, to create a D-loop. The 3' end of the invading DNA strand can be extended by DNA polymerase. If the invading strand is then separated from the invaded DNA, it can be joined to its cognate broken end via strand annealing. Replication and DNA ligation completes the repair process.

Although SDSA may be the most common pathway for double strand break repair, other variants either exist or have been proposed. All variants share the key steps of rendering a DNA single stranded by the action of helicases and nucleases, DNA strand invasion promoted by a RecA-family recombinase, extension of the invading DNA 3' end with a DNA polymerase, and final ligation of nicks with DNA ligase.

What is needed in the art is a system for efficient repair of DNA double-strand breaks in vitro. Such a system will benefit multiple areas such as DNA genotyping in forensic science, DNA extraction from ancient sources, genome sequencing and metagenomics.

## BRIEF SUMMARY OF THE INVENTION

The present invention relates generally to a method for joining two DNA fragments in vitro, both of which possess homologous DNA sequences, through a simplified process of DNA double-strand break repair. The method requires three proteins, RecA protein, the single-stranded DNA binding protein (SSB) and DNA polymerase I or homologues of these proteins.

In one embodiment, the present invention is a method of repairing a DNA double-strand break in vitro comprising the steps of: (a) providing a duplex DNA molecule wherein the molecule has a double-strand break and wherein the molecule is not super-coiled; (b) providing a single-stranded DNA (ssDNA) targeting fragment, wherein the targeting fragment is homologous to at least 15 nucleotides of the DNA molecule of step (a) (or providing a targeting fragment in a double-stranded form and converting the targeting fragment to single-strand DNA); (c) adding RecA protein or RecA protein homologue to the targeting fragment; (d) adding single-stranded DNA binding protein (SSB) or SSB homologue; (e) adding the DNA molecule of step (a) to the mixture resulting from step (d) and incubating until a strand invasion of the targeting fragment into the DNA molecule of step (a) has occurred; and (f) adding DNA polymerase and dNTPs, and incubating until an extended DNA comprising sequences from the targeting fragment and the DNA molecule of step (a) has been produced such that purification of the lengthened DNA is possible.



Preferably, step (c) is in the presence of a suitable buffer containing an ATP regenerating system. Preferably, the RecA protein and/or RecA homologue, SSB and/or SSB homologue, and DNA polymerase are from bacterial sources. More preferably, the RecA protein and/or RecA homologue, SSB and/or SSB homologue, and DNA polymerase are isolated from *Escherichia coli*.

In a preferred embodiment of the invention, the DNA polymerase is selected from the group consisting of DNA polymerase I, DNA polymerase V, phi29 DNA polymerase and engineered translesion synthesis DNA polymerases. Most preferably, the DNA polymerase is DNA polymerase I.

In another embodiment, the present invention is a kit for DNA double-strand break repair in vitro comprising an effective amount of (a) RecA protein or RecA protein homologue, (b) SSB or SSB homologue and (c) DNA polymerase. Preferably, the kit additionally comprises a suitable buffer for RecA protein or RecA homologue, containing an ATP regenerating system.

In another embodiment, the present invention is a method of repairing DNA double-strand breaks for forensic DNA genotyping comprising the steps of: (a) preparing at least a pair of single-stranded DNA fragments, wherein 3' end of each single-stranded DNA of a pair is proximal to an analyzed target region, with one single-stranded DNA fragment encompass homology to one side of the analyzed target region and the other single-stranded DNA fragment encompass homology to the opposite side of the analyzed target region; (b) adding RecA protein or RecA protein homologue to the single-stranded DNA; (c) adding SSB or SSB homologue; (d) adding a forensic, non-supercoiled DNA sample with a double-strand break and incubating until a strand invasion of the targeting fragments into the forensic DNA molecule has occurred; (e) adding DNA polymerase and dNTPs, and incubating until the invading DNA has been extended so as to comprise sequences from the targeting fragment and the forensic DNA molecule and until amplification or purification of the lengthened DNA is possible; and (f) analyzing the product of the reaction as part of a forensic DNA genotyping procedure. Preferably, the single-stranded DNA fragments encompass 15-2000 bp homology to regions flanking the analyzed target region. Even more preferably, the single-stranded DNA fragments encompass 150-400 bp homology to regions flanking the analyzed target region. Most preferably, the single-stranded DNA fragments encompass 200-400 bp homology to regions flanking the analyzed target region.

In one embodiment of the invention, the single-stranded DNA of step (a) is directly synthesized by standard oligo-